

SALAMARKER: A CODE GENERATOR AND STANDARDIZED MARKING SYSTEM FOR USE WITH VISIBLE IMPLANT ELASTOMERS

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Abstract.—Visible implant elastomers (VIEs) provide lasting tags for small animals such as salamanders that are otherwise difficult to mark. Despite the widespread use of VIEs, few standardized coding systems have been proposed, and those that do exist are applicable to a limited number of species. Without such standardized systems, implementing and documenting VIE codes remains complicated and time consuming. To assist with future studies that use VIEs, we present a system of code nomenclature and a computer program that will generate lists of unique marks based on the users' parameters. This system of denoting VIE combinations coupled with a new code generator will save researchers time and effort while preventing errors inherent in creating independent coding schemes.

Key Words.—amphibians; computer program; mark; mark recapture; reptiles; salamander; VIE

INTRODUCTION

Applying unique marks to individuals in ecological studies allows for estimation of population size and demographic parameters (such as birth, death, and survival rates) as well as movement, home range size, and individual growth rates (Krebs 1989; Donnelly and Guyer 1994). Accurate estimates require that marks are not lost or misread and have no effect on recapture rates (Krebs 1989). Ideal marks are cheap, easy to use in laboratory and field settings, adaptable to organisms of different sizes, and cause minimal pain and stress to the organism (Ferner 2007).

A wide variety of techniques have been used to mark herpetofauna (for complete reviews see Donnelly and Guyer 1994; Ferner 2007). As a result, many of the traditional marking techniques, such as shell notching in turtles (Cagle 1939; Ernst et al. 1974), scale clipping in snakes (Blanchard and Finster 1933; Brown and Parker 1976), and toe clipping in amphibians (Martof 1953; Twitty 1966; Hero 1989; Waichman 1992), have standardized coding systems to facilitate quick, easy, and reliable data recording. Relatively new marking techniques such as visible implant elastomers (VIEs; Northwest Marine Technology, Inc., Shaw Island, Washington, USA) however, often lack standardized coding systems despite their extensive use in the field (Table 1).

VIE marks may be applied in different colors and body locations to create unique combinations that may be used as batch marks or individual marks. The number of available combinations depends on the number of colors, body locations, and tags per individual (here "tag" refers to a single VIE injection while "mark" refers collectively

to all tags on an individual). These values can be calculated with the formula:

$$\left(\frac{L!}{(L-N)!N!}\right)C^N$$

where L is the number of body locations used, N is the number of tags per individual, and C is the number of colors used (Northwest Marine Technology. 2008. Visible implant elastomer tag project manual. Available from <http://www.nmt.us/products/vie/vie.shtml> [Accessed 22 March 2010]). For example, with six possible body locations, three tags per individual, and four colors, there are 1280 unique combinations available. Listing possible combinations of colors and body locations can be complicated and time consuming. To this end, standardizing such systems will save time and reduce errors, especially in studies of herpetofauna with large sample sizes.

The literature is well-populated with investigations into the retention and readability of VIE marks in a variety of amphibian and reptile species (Table 1), but few studies provide practical suggestions for the actual implementation of a VIE coding scheme. Hoffman et al. (2008) developed an alpha-numeric marking system for anurans that combines a single toe clip with VIE tags injected at the toes, thus creating the potential for thousands of unique marks. This VIE and toe clip method, or 'VIE-C' method, reduces the number of toe clips per individual compared to traditional toe clipping systems, which are criticized for having potential negative effects on survivorship and behavior (Davis and Ovaska 2001; Parris and McCarthy 2001). However, the VIE-C technique still requires that a toe is clipped and

TABLE 1. Studies that use and or evaluate use of visible implant elastomers (VIEs) to mark a variety of reptile and amphibian species and age classes.

Taxa	Species	Type of Mark	Reference
Lacertilia	<i>Neoseps reynoldsi</i>	Individual	Penney et al. 2001 ¹
	<i>Anolis carolinensis</i>	Individual	Irschick et al. 2006
	<i>Anolis sagrei</i>	Individual	Calsbeek and Irschick 2007
Serpentes	<i>Pantherophis guttatus</i>	Batch	Hutchens et al. 2008 ¹
Anura	<i>Lithobates sylvaticus</i>	Batch	Vasconcelos and Calhoun 2004
	<i>Lithobates sylvaticus</i>	Batch	Moosman and Moosman 2006 ¹
	<i>Lithobates sylvaticus</i> ²	Batch	Grant 2008 ¹
	<i>Hyla chrysoscelis</i>	Batch	Pittman et al. 2008
	<i>Pelophylax esculentus</i> ² , <i>P. lessonae</i> ² , <i>Rana temporaria</i> ²	Individual	Anholt et al. 1998 ¹
	<i>Pelophylax esculentus</i>	Individual	Nauwelaerts et al. 2000 ¹
	<i>Hyla</i> spp., <i>Osteopilus septentrionalis</i>	Individual	Hoffman et al. 2008 ¹
	<i>Hyla</i> spp., <i>Osteopilus septentrionalis</i>	Individual	Campbell et al. 2009 ¹
	<i>Ambystoma maculatum</i>	Batch	Vasconcelos and Calhoun 2004
	<i>Desmognathus fuscus</i> , <i>D. monticola</i>	Batch	Kinkead et al. 2006 ¹
Caudata	<i>Ambystoma maculatum</i> , <i>A. talpoideum</i>	Batch	Kinkead and Otis 2007
	<i>Eurycea nana</i>	Batch	Phillips and Fries 2009 ¹
	<i>Plethodon vehiculum</i>	Individual	Davis and Ovaska 2001 ¹
	<i>Eurycea bislineata wilderae</i>	Individual	Bailey 2004 ¹
	<i>Plethodon cinereus</i>	Individual	Heemeyer et al. 2007 ¹
	<i>Desmognathus fuscus</i> ² , <i>Eurycea bislineata</i> ²	Individual	Grant 2008 ¹

¹Study evaluates use of VIE marking technique

²Larval individuals marked

that the target organism's toes are large enough to receive a single VIE injection. This method is not applicable to organisms with small toes or lacking toes, such as small salamanders and anurans, amphibian larvae, fish, and crustaceans.

Herein we describe a simple and efficient way of denoting VIE marks and a free computer program that can quickly generate lists of codes specific to the users' needs. We do not present the use of VIEs as a new marking method, as this technique has previously been used and tested in numerous studies (Table 1); rather, we seek to improve code nomenclature and generation. Our code nomenclature is applicable to any animal that can be marked with VIE, and our program should save time and avoid errors in code generation, as well as provide more user options than existing programs.

PROPOSED MARKING SYSTEM

VIE coding scheme.—An efficient VIE coding system requires a simple way of denoting each mark and a systematic way of producing extensive lists of these marks. The use of colors in VIE precludes the creation of a purely numeric coding system like those developed for scute notching (Cagle 1939) and toe clipping (Martof 1953; Twitty 1966; Hero 1989). Thus, an alphanumeric system is more appropriate. The alphanumeric code developed for toe clipping by Waichman (1992), in which limbs are represented by letters and toes by numbers, can be modified for use in VIE by changing

the letters to represent colors and using numbers to represent body locations.

Small salamanders provide an example of how this code nomenclature can be applied. In the case of small salamanders, VIEs may be applied in up to six locations on the salamander's ventral side: posterior to the base of the forelimbs, anterior to the base of the hind limbs, and posterior to the base of the hind limbs (Davis and Ovaska 2001; Heemeyer et al. 2007). With these locations numbered (as in Fig. 1), R1B4 represents an individual with one red VIE tag posterior to the base of the left forelimb and one blue VIE tag anterior to the base of the right hind limb (left and right here refer to the observer's left and right when viewing the animal's ventral side). This system can easily be modified for any animal as long as the body locations are numbered. We note that in choosing body locations for VIE injection, it is important to conduct pilot studies to ensure marks are not obscured by dark pigment and do not migrate or break apart in laboratory or field settings (Davis and Ovaska 2001; Moosman and Moosman 2006; Heemeyer et al. 2007); our discussion of all possible locations for application does not imply that all may work for all species or in all situations.

Code generator: SalaMarker.—It is difficult to quickly and systematically generate long lists of codes by hand, and such lists are prone to human error. We know of only one computer program developed for generating VIE codes. Northwest Marine Technology

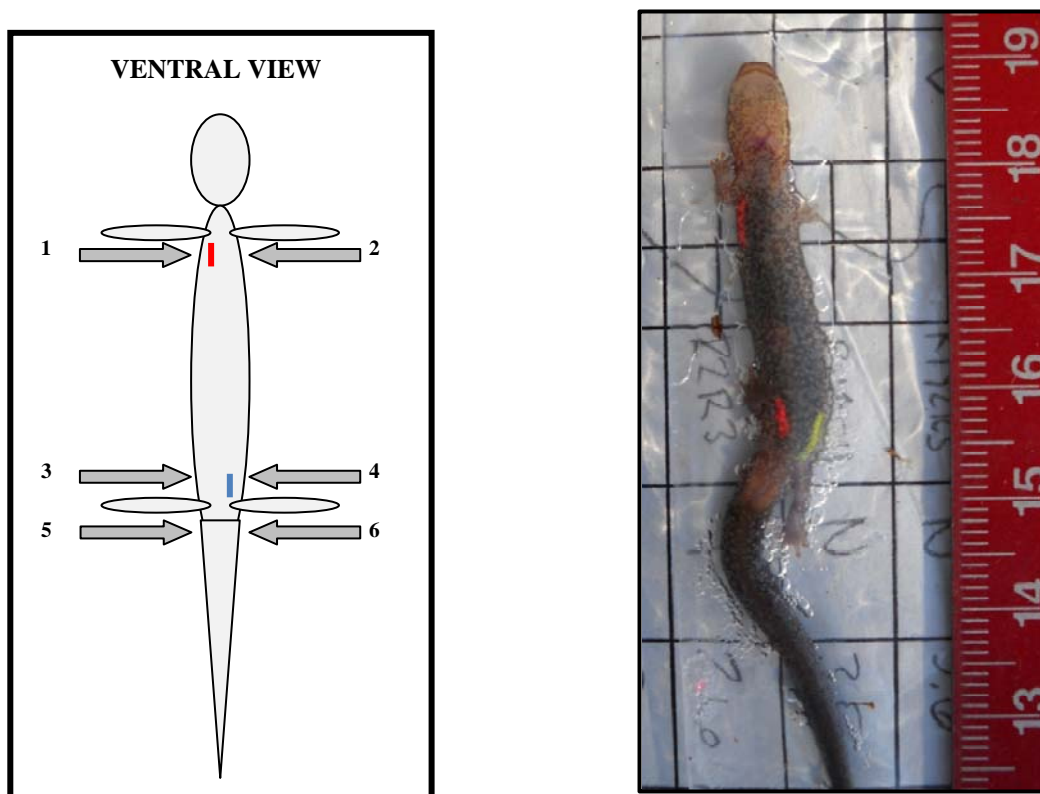


FIGURE 1. Visible implant elastomer (VIE) marking locations for salamanders. The diagram on the left shows an example mark denoted as R1B4. On the right is an Eastern Red-backed Salamander (*Plethodon cinereus*) injected with red and yellow VIE tags to form the mark R1R3Y4 (Photographed by Jami E. MacNeil).

(NMT), Inc., created a VIE Color Code Generator (available at: <http://www.nmt.us/support/software/viecodes/viecodes.shtml>) that provides the number of possible combinations given the chosen parameters and uses those parameters to generate a list of these combinations. The program is accurate and easy to use, but suffers from several limitations: (1) the number of tags per specimen must remain constant (i.e., may not vary from one to the maximum desired N); (2) the number of tags per specimen may not exceed three; (3) the number of body locations may not exceed 10; (4) the codes are written out in a long form that takes up space; and (5) the output is a portable data file (.pdf) that can be printed but not copied and pasted into other programs such as Microsoft Excel.

We present a new computer program that addresses these limitations. Like the NMT code generator, this program creates an output of all possible codes based on user input of parameters. The list of codes is defined by the user's preference of body locations, number of tags per individual (cumulative or constant), and any number and combination of colors. When the number of tags per individual is cumulative, the code output will include all marks with the lowest number of tags per individual,

followed by all marks with the next lowest number of tags per individual and so on until the specified number of desired marks is reached. When the number of tags per individual is constant, all marks in the output file will contain the same number of tags. The option of a cumulative tagging method extends the number of available combinations when all else is equal, though a drawback to this is discussed below. The program prompts the user to enter each parameter in a step-by-step fashion. The final window displays all entered parameters and the number of codes generated (Fig. 2). The corresponding output is given in a text (.txt) file (Fig. 3) in the folder where the program is stored. The number of body locations may range from one to 99, and tags per individual is constrained only by the chosen number of body locations. This feature will be useful for study animals with more than 10 potential body locations (Curtis 2006). Codes are presented in a space-efficient 'DYE-WORD' such as R1B4, which fits easily on a datasheet and allows hundreds of codes to fit on a single sheet of paper. The output file may be copied into programs such as Microsoft Excel and Word and printed for use in the field or lab.

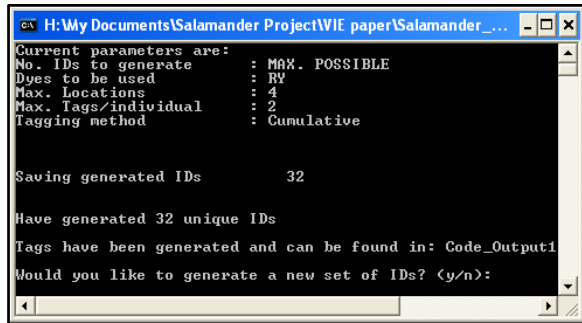


FIGURE 2. Example interface of the program SalaMarker. The final screen displays all entered parameters and the number of generated codes. In this example, the program created the maximum number of codes possible given two colors (red and yellow), four body locations, and one to two tags per individual (maximum of two tags per individual, cumulative).

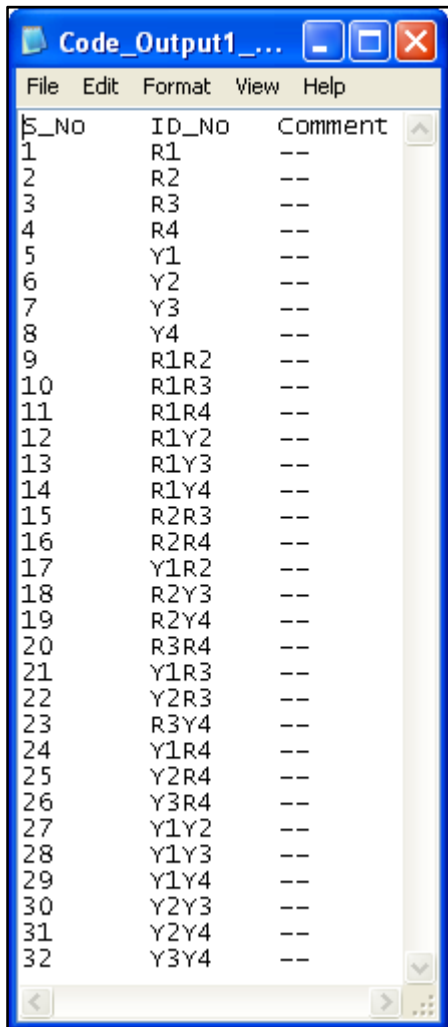


FIGURE 3. Example code output (.txt file) from the program SalaMarker. Given two colors, four body locations, and up to two tags per individual, 32 unique identifying marks are possible.

Recommendations.—The ‘cumulative’ tagging method option in the computer program allows the number of tags per individual (in the equation, N) to vary within a study. It is important to note there is a greater risk of misreading recaptures when N is not constant. For example, when a recapture is recorded as having two VIE tags, it may not be certain that the individual did not originally have three tags, with the third being either lost or overlooked by the observer. When all individuals are marked with the same number of tags, the observer always knows if a tag is missing or overlooked. Tag retention for VIE is relatively high in field (Davis and Ovaska 2001) and lab studies (Davis and Ovaska 2001; Bailey 2004; Heemeyer et al. 2007), but tags may be difficult to see if they are small, injected too deeply, or consist of one of the less visible colors. We suggest researchers use their discretion in deciding whether to let N vary, thereby reducing handling time and stress to the individuals that receive fewer tags, or to keep N constant to avoid errors in reading recaptures.

This system of denoting VIE combinations and the computer program to generate lists of relevant marks may save researchers time and effort spent creating such a code from scratch and may prevent errors that result from manually entering letter codes into a spreadsheet. Use of a standardized code may aid in data sharing and facilitate collaborative research. The program’s capacity for a wide range of parameter values and the user’s ability to manipulate output files should assist researchers in using VIEs to mark animals. This code was developed for herpetofauna but can be applied to any organism in which one uses an alphanumeric system. The program is available for free online at: <http://web.ics.purdue.edu/~rodw/> under the heading SalaMarker. A help file is provided with the program to facilitate ease of use.

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